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EXAMINER	
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ART UNIT	PAPER NUMBER
1636	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/690,880	LEE ET AL.	
	Examiner	**Art Unit**	
	Jennifer Dunston	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 February 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *49-55,57,61-64,96 and 98-106* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *49-55,57,61-64,96 and 98-106* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *22 October 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *Appendices I-III*.

DETAILED ACTION

The finality of the Office action mailed 9/29/2009 is withdrawn.

Receipt is acknowledged of an amendment, filed 2/12/2010, in which claims 11-18, 20-48, 65-93, 97 and 107 were cancelled, and claims 49-51, 54, 55, 57, 96, 98, 100 and 102 were amended. The amendment has been entered. Claims 49-55, 57, 61-64, 96 and 98-106 are pending.

Election/Restrictions

Applicant elected Group III with traverse in the reply filed 6/21/2006. Applicant further elected the combination of biomarkers comprising SEQ ID NOs: 1, 2, 5, 15 and 16 (IL-8, COX-2, SAA1, PPAR-alpha and PPAR-gamma, respectively), and the oligonucleotide primers comprising SEQ ID NOs: 45 and 46, which amplify SEQ ID NO: 1; SEQ ID NOs: 47 and 48, which amplify SEQ ID NO: 2; SEQ ID NOs: 53 and 54, which amplify SEQ ID NO: 5; SEQ ID NOs: 73 and 74, which amplify SEQ ID NO: 15; and SEQ ID NOs: 75 and 76, which amplify SEQ ID NO: 16.

In the Office action mailed 9/11/2006, the Examiner withdrew the restriction requirement between Groups III and V. Although the restriction requirement mailed 5/5/2006 required an election of a single invention, which is one combination of sequences, the record indicates that claims reading on less then the full combination of sequences have been examined. Thus, the claims will be considered as they read at least two sequences selected from the group consisting of SEQ ID NOs: 1, 2 and 5, as well as sequences selected from SEQ ID NOs: 15 and 16. The other combinations of sequences remain withdrawn.

Claims 49-55, 57, 61-64, 96 and 98-106 are under consideration.

Claim Objections

Claim 49 is objected to because of the following informalities: at line 8, the word "and" should be deleted. The word "and" should be placed at the end of line 11. Claims 50-55, 57, 61-64 and 96 depend from claim 49 and are objected to for the same reason. Appropriate correction is required.

Claim 96 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 96 requires the biomarker to comprise (i) SEQ ID NO: 1, (ii) SEQ ID NO: 2 <u>or</u> (iii) SEQ ID NOs: 1 and 2. However, the independent claim from which claim 96 ultimately depends requires the biomarkers to comprise SEQ ID NOs: 1 <u>and</u> 2. SEQ ID NO: 1 or 2 is not required by some embodiments of claim 96, and those embodiments are broader in scope than the independent claim such that claim 96 does not necessarily include every limitation of the independent claim.

Claims 50 and 99 are objected to because of the following informalities: the claim reads on non-elected invention. Appropriate correction is not required at this time.

Claim 98 is objected to because of the following informalities: at line 9, the word "and" should be deleted. The word "and" should be placed at the end of line 11. Claims 99-106 depend from claim 98 and are objected to for the same reason. Appropriate correction is required.

Response to Arguments - Claim Objections

The objection of claim 97 is moot in view of Applicant's cancellation of the claim in the reply filed 2/12/2010.

The previous objections of claims 49, 51-55, 57, 61-64 and 96 have been withdrawn in view of Applicant's amendment to the claims in the reply filed 2/12/2010.

The objection of claims 50 and 99 are maintained, because the independent claims are not allowable. Thus, all sequences are not eligible for rejoinder at this time.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 98-106 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This is a new rejection.

Claim 98 is vague and indefinite in that the metes and bounds of the claimed method are unclear. The preamble recites "method for assessing the risk of colorectal cancer." However, the last lines of the claim recite, "wherein an increase in at least SEQ ID NO: 1 and/or 2 is indicative of colorectal cancer." The metes and bounds of the phrase "indicative of colorectal cancer" are unclear. It is unclear if the phrase is referring to an indication of increased risk, or whether it refers to an indication of the presence of colorectal cancer (i.e., diagnosis). The specification envisions both aspects with regard to diagnosis and risk assessment (e.g. paragraph

[0029]). Therefore, it is unclear if one necessarily accomplishes what is intended for the method. It would be remedial to amend the claim language to clearly indicate that the method is "indicative of increased risk of colorectal cancer."

Claims 99-106 depend from claim 98 and are rejected for the same reasons applied to claim 98.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 98-106 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method where an increase in expression of at least SEQ ID NO: 1 and/or 2 is indicative of an increased risk for colorectal cancer, does not reasonably provide enablement for diagnosing colorectal cancer. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. This is a new rejection.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art, the amount of experimentation necessary and the relative skill levels of those in the art. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Nature of the Invention: Claims 98-106 are drawn to a method for assessing the risk of colorectal cancer, comprising (i) selecting a panel of biomarkers comprising polynucleotides having sequences including SEQ ID NOs: 1 and 2; (ii) obtaining a biological colorectal sample from a subject; (iii) isolating cellular RNA from the sample; (iv) amplifying and quantifying RNA expression levels in a biological colorectal sample from a subject for each biomarker in the panel including SEQ ID NOs: 1 and 2; (v) comparing the quantified expression levels of each biomarker in the sample to each of the same biomarker expression level in a normal control colorectal sample; and (vi) determining a difference in the expression levels of the biomarkers in the panel including SEQ ID NOs: 1 and 2 in the biological sample compared to the normal control, wherein an increase in at least SEQ ID NO: 1 and/or 2 is **indicative of colorectal cancer**.

The invention is complex in that if "indicative of colorectal cancer" is interpreted as diagnosis of colorectal cancer, the invention involves measuring a change in the level of RNA by amplification, such that a diagnosis of colorectal cancer is made. The present specification teaches that the two criteria for assessing the effectiveness of biomarkers are selectivity and sensitivity, where selectivity refers to the percentage of patients correctly diagnosed, and sensitivity is defined as the probability that the disease is detected at a curable stage (e.g., paragraph [0014]). The specification teaches that there is a difference between diagnosis and risk assessment. The specification states the following at paragraph [0029]:

> The difference between risk assessment and early detection is the degree of certainty regarding acquiring CRC. Biomarkers that are used for early detection confer less than 100% certainty of CRC within a time interval, whereas biomarkers used for early detection confer an almost 100% certainty of the onset of the disease within a specified time interval.

If diagnosis is required, the biomarkers must confer an almost 100% certainty of the onset of the disease within a specified time interval.

Breadth of the claims: The claims may be interpreted as requiring the diagnosis of colorectal cancer, which complicates the invention.

Guidance of the specification/The existence of working examples: The specification discloses the use of a mouse multiple intestinal neoplasia (MIN) model to determine expression differences between mouse MIN subjects comprising a chemically induced mutation in the APC gene and normal control littermates for which there was not aberration of the APC gene (page 5, paragraph 18). From these studies candidate genes were selected for study in human subjects; and from these studies with human samples, a disclosed panel of biomarkers was obtained. In one disclosed example, a panel of six biomarkers is used "as the basis for determination of CRC in human subjects" -- although the biomarkers were applied to samples obtained from patients known to have CRC and from individuals validated as normal controls (page 8, paragraph 27). The results are shown in Figure 2B for the panel of six markers, which include IL-8 (SEQ ID NO: 1), and COX2 (SEQ ID NO: 2), but does not include the markers of SEQ ID NO: 5, 15 or 16. In another example, multiple biopsy samples taken from one exemplary patient diagnosed with CRC showed differences in expression of three biomarkers (see paragraph bridging pages 9 and 10). In the last example, the specification teaches that multiple biopsies (again from a single patient), taken over a 53 cm region of the colon, where able to "distinguish differences in the colon tissue for the patient" whereas the same biopsy samples were rendered normal by conventional histological analysis. The specification teaches that such results demonstrate a

minimally invasive swabbing collection method from an area distant from a cancerous lesion is capable of indicating a "non-normal colon condition" (page 10, paragraph 32).

The specification does not provide a working example of the claimed method where a subject is diagnosed with colorectal cancer based upon the RNA expression levels of SEQ ID NOs: 1 and/or 2.

In addition, it is acknowledged in the specification that "there is a distinct difference between research on a specific a gene, its expression, protein product, and regulation, and understanding what genes are critical to include in a panel used to for the analysis of CRC that is useful in the management of patient care for the disease." (paragraph [0017]) and the application demonstrates that there is substantial variation in expression levels of individual genes when compared with control sample (paragraph [0027]), which necessitates the use of a panel of biomarkers for diagnostic validity.

State of the prior art and level of predictability in the art: The "predictability or lack thereof" in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. If one skilled in the art can readily anticipate the effect of a change within the subject matter to which the claimed invention pertains, then there is predictability in the art. On the other hand, if one skilled in the art cannot readily anticipate the effect of a change within the subject matter to which that claimed invention pertains, then there is lack of predictability in the art. Accordingly, what is known in the art provides evidence as to the question of predictability.

The physiological art is recognized as unpredictable. (MPEP 2164.03.) In cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides

broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws. In cases involving unpredictable factors, such as most chemical reactions and physiological activity, the scope of enablement obviously varies inversely with the degree of unpredictability of the factors involved.

In the instant case, the specification teaches that "given the complexity of biological systems, discovery of panels useful in providing value in patient care management for CRC is in the nascent stage" (page 5, paragraph [0016]). The prior art supports this statement.

In general, the prior art teaches that there are many factors that need to be considered in order to develop a reliable genetic test. The art teaches that before a putative biomarker can be used as a surrogate endpoint it must be validated as such. Wagner (*Dis. Markers* 18:41-46, 2002, of record) acknowledges in the Abstract, "Putative biomarkers are typically identified because of a relationship to known or hypothetical steps in a pathophysiologic cascade. Biomarker discovery can also be effected by expression profiling experiment using a variety of array technologies and related methods." However, Wagner cautions, "A rational basis for recommending the use of a putative biomarker does not guarantee the utility of the biomarker or its qualification as a surrogate endpoint" (paragraph bridging the left and right columns on page 43) and "Biomarkers require validation in most circumstances" (paragraph bridging pages 43-44).

Frank *et al.* (*Nature Rev.* 2:566-580, 2003, of record) concurs, stating, "The standard concepts of test-re-test reliability and validity apply with equal force to clinical biomarkers as they do in any assay system" and, "The work required to establish the reliability and validity of a

new biomarker should not be underestimated in general, and in particular needs of planning for each combination of clinical indication and mechanism of action" (paragraph bridging the left and right columns on page 568). Feng *et al.* (*Pharmacogenomics* 5:709-719, 2004, of record) teaches, "The development and validation of clinically useful biomarkers from high-dimensional genomic and proteomic information pose great research challenges. Present bottle necks include: that few of the biomarkers showing promise in initial discovery were found to warrant subsequent validation...A molecular profiling approach, although promising, has a high chance of yielding biased results and overfitted models" (Abstract).

The unpredictability of correlating gene expression level to any phenotypic quality is also supported by the teachings of Wu (*J. Pathol.* 195(1):53-65, 2001, of record). Wu teaches that gene expression data must be interpreted in the context of other biological knowledge, involving various types of "post genomics" informatics, including gene networks, gene pathways, and gene ontologies (page 53, left column). The reference indicates that many factors may be influential to the outcome of data analysis, and teaches that expression data can be interpreted in many ways. The conclusions that can be drawn from a given set of data depend heavily on the particular choice of data analysis. Much of the data analysis depends on such low-level considerations as normalization and such basic assumptions as normality (page 63 – Discussion).

Additional post filing art reveals that most gene association studies are typically wrong. Lucentini (The Scientist, Vol. 18, page 20, 2004, of record) teach that it strikingly common for follow-up studies to find gene-disease associations wrong (e.g. page 2, 1st paragraph). Lucentini teaches that two recent studies found that typically when a finding is first published linking a given gene to a complex disease there is only roughly a one-third chance that the study will

reliably confirm the finding (e.g. page 2, 3rd paragraph). Lucentini teaches that bigger sample sizes and more family-based studies, along with revising statistical method, should be included in the gene association studies (e.g. page 3, 2nd paragraph).

The art teaches that gene expression analysis is commonly used for three different purposes: (1) as a screening tool to identify individual genes of interest that might contribute to an important biological function, (2) to obtain insight into an important biological function, and (3) as a classification tool to sort cases into clinically important categories (Pusztai and Hess, Annals of Oncology, Vol. 15, pages 1731-1737, 2004, of record; e.g., paragraph bridging pages 1732-1733). In the instant case, the specification uses gene expression analysis to as a screening tool to identify genes of interest, and to obtain insight into an important biological function. However, the claims are drawn to using gene expression analysis to diagnose colorectal polyps and colorectal carcinoma. Pusztai and Hess teach that validation of gene expression important to biological function may be validated by using different methods, such as RT-PCR, whereas the most appropriate validation for using gene expression analysis as a classification tool is testing the predictor on independent sets of cases (e.g., page 1733, left column, 1st full paragraph). In the instant case the specification and declaration of Dr. Lee, filed 12/27/2007, provide an analysis by RT-PCR, but do not test the predictor on a set of cases.

Further, Shalon et al (US 2001/0051344 A1, Dec 13, 2001, of record) teach that due to variations in genetic make-up of unrelated individuals in a heterogeneous society, differences in the expression of a gene between any two individuals may or may not be significant (e.g., paragraph [0155]). Shalon et al further teach that the larger the number of individuals tested, the more significant the remaining differences in gene expression become and samples from at least

5 and preferably 20-50 different test individuals are assayed to obtain statistically meaningful data showing a statistical elevation or reduction in report levels when compared to control levels (e.g., paragraph [0156]). Pusztai and Hess teach that larger samples sizes may be needed to validate classification tests, and the number of samples will vary depending upon the acceptable error rates, level of inter-patient variability, the size of the difference in mean expression values, and the prevalence of the phenotype among the group being tested (e.g., page 1734, paragraph bridging columns; Table 1).

The prior art reveals that differences in gene expression observed between two groups are do not necessarily provide markers that can be used to reliably classify a subject. Golub et al (Science, Vol. 286, pages 531-537, October 1999, of record) teach the use of a two-step procedure to test the validity of gene expression levels as predictors: step 1 involves cross-validation of the predictors on the initial data set, where one withholds a samples, builds a predictor based only on the remaining samples and predicts the class of the withheld sample; step 2 involves the repetition of assessing the clinical accuracy of the predictor set on an independent set of samples (e.g., page 532, right column). Although Golub et al could detect gene expression differences between chemotherapy responders and non-responders, those differences could not be use to predictably classify individuals (e.g., page 533, paragraph bridging left and middle columns). Accordingly, the art demonstrates the unpredictable nature of extrapolating gene expression differences to a method of class prediction.

Thus, the state of the art is underdeveloped with respect to the use of nucleic acids to diagnose and manage disorders in general.

Relative skill of those in the art and quantity of experimentation needed to make or use the invention: Although the level of skill in the art is high, one of ordinary skill would not be able to make and use the full scope of the invention now claimed and as asserted in the application without undue experimentation. The specification discloses a single panel of genes exhibiting altered expression in a mouse model comprising a chemically induced mutation in the APC gene and normal control littermates for which there was not aberration of the APC gene and analysis of a panel of six biomarkers applied to samples obtained from patients known to have CRC and from normal controls. The application also discloses differences in expression of the biomarkers in biopsy samples taken from one exemplary patient diagnosed with CRC. Based on this disclosure, the application seeks to claim a method comprising measuring expression of any panel of biomarkers comprising any two polynucleotides including SEQ ID NO: 1 and 2 expressed in a biological colorectal sample and comparing such expression levels to control biological colorectal sample, wherein the comparison may be indicative (i.e., diagnostic) of colorectal cancer or may be used to assess risk of colorectal polyps and colorectal cancer. A large amount of experimentation would be required to determine if the gene expression levels from a single test subject can be used to reliably categorize the individual as having or not having colorectal cancer.

In view of the foregoing, the skilled artisan would not be able to make and use the invention presently claimed without first engaging in undue experimentation. Therefore, the claims are properly rejected under 35 USC § 112, first paragraph, as lacking a fully enabling disclosure.

Response to Arguments - 35 USC § 112

The rejection of claims 79 and 81-88 under 35 U.S.C. 112, first paragraph (new matter) is moot in view of Applicant's cancellation of the claims in the reply filed 2/12/2010.

The rejection of claims 97 and 107 under 35 U.S.C. 112, first paragraph (scope of enablement) is moot in view of Applicant's cancellation of the claims in the reply filed 2/12/2010.

The rejection of claims 49-55, 57, 61-64 and 96 under 35 U.S.C. 112, first paragraph (scope of enablement) has been withdrawn. However, upon further consideration, a new ground(s) of rejection is made under 35 U.S.C. 103(a).

With respect to the rejection of claims 98-106 under 35 U.S.C. 112, first paragraph (scope of enablement), as presented above, Applicant's arguments filed 2/12/2010 have been fully considered but they are not persuasive. The response asserts that the objection has been obviated by the amendment to the claims. However, claim 98 recites the phrase "wherein an increase in at least SEQ ID NO: 1 and/or 2 is indicative of colorectal cancer." Although the method is directed to "assessing risk of colorectal cancer," the phrase "indicative of colorectal cancer" may be interpreted as being directed to the diagnosis of colorectal cancer. Thus, a rejection under 35 U.S.C. 112, first paragraph, has been made to address this interpretation of claims 98-106.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 49, 54, 55, 57, 61, 63, 64, 96, 98, 100-103, 105 and 106 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kanaoka et al (Japanese Journal of Gastroenterology, Vol. 99, No. 44taikai, page A634, Abstract P-379, September 20, 2002; see the entire reference and attached translation) in view of GenBank Accession No. XM_051900 (GI: 14730543, publicly available July 2001; see the entire reference), Brew et al (European Journal of Cancer, Vol. 32A, No. 12, pages 2142-2147, 1996; see the entire reference), di Celle et al (Blood, Vol. 84, No. 1, pages 220-228, July 1994; see the entire reference), and GenBank Accession No. XM_031289 (GI: 14731054, publicly available July 2001; see the entire reference). This is a new rejection.

For the purpose of this rejection, independent claims 98 and claims that depend therefrom have been interpreted as being directed to a method of assessing the risk of colorectal cancer (see the rejection under 35 U.S.C. 112, second paragraph).

Kanaoka et al teach a method comprising (i) selecting a panel of biomarkers comprising CEA and COX-2; (ii) obtaining colorectal cells from feces samples of subject with colorectal cancer and subjects with no organic disease of the colon; (iii) isolating cellular RNA from the feces samples; (iv) amplifying and quantifying RNA expression levels of CEA, COX-2, and β-actin; (v) comparing the quantified expression levels of CEA and COX-2 in the samples obtained from subjects with colorectal cancer to samples obtained from subjects with no organic disease of the colon, wherein an increase in COX-2 expression correlates with an increased risk of colorectal cancer (see the entire translation). Kanaoka et al teach that the RT-PCR amplification products were identified by electrophoresis on a 4% agarose gel (see the entire translation). Kanaoka et al teach that COX-2 RNA expression was detected in 15 of the 20 patients with colorectal cancer and none of the patients with no organic disease of the colon, resulting in a sensitivity of 75% and specificity of 100% (see the entire translation).

Kanaoka et al do not teach that Cox-2 has the sequence of SEQ ID NO: 2. Kanaoka et al do not teach amplifying, quantifying and comparing the expression of SEQ ID NO: 1 (IL-8) in the colorectal samples. Kanaoka et al do not explicitly teach the labeling of the amplified cDNA with a chromophore.

GenBank Accession No. XM_051900 teaches the sequence of human Cox-2 (a.k.a. prostaglandin-endoperoxide synthase 2). This sequence is identical to instant SEQ ID NO: 2 (see the attached alignment in Appendix I).

Brew et al teach that IL-8 production was known to be a feature of certain human tumor cell lines derived from colon carcinoma (e.g., page 2143, left column, 1st paragraph). Brew et al teach the collection of tissue samples of normal colon, neoplastic colorectal and adjoining non-

involved tissue, and lymph node metastasis (e.g., page 2143, left column, 2nd paragraph). Brew et al teach the construction of sense and antisense riboprobes to the IL-8 cDNA for use in *in situ* hybridization to quantify the level of IL-8 RNA expression in the collected tissue samples (e.g. page 2143, left column, 4th paragraph and right column, 1st paragraph). Brew et al teach that all 10 adenocarcinoma samples, including a lymph node metastasis, showed cytoplasmic hybridization with the IL-8 probe, and the intensity of staining ranged from weak to strong (e.g., paragraph bridging pages 2143-2144; Table 1). Further, Brew et al compared the level of IL-8 RNA expression in the adenocarcinomas to the expression in normal colonic mucosa, and determined that expression was increased in the adenocarcinoma relative to the normal colonic mucosa, which showed much weaker staining or was completely negative (e.g., paragraph bridging pages 2143-2144).

Di Celle et al teach a sensitive reverse-transcriptase polymerase chain (RT-PCR) analysis performed to evaluate the expression of a panel of cytokine mRNAs in unstimulated B-LL cells, where the panel includes IL-8 (e.g., page 220, paragraph bridging columns). Di Celle et al teach RT-PCR of IL-8 from total cellular RNA followed by analysis in gels stained with ethidium bromide (e.g., page 221, right column, full paragraph). Di Celle et al teach that the IL-8 mRNA can be quantified relative to a co-amplified gene (e.g., paragraph bridging pages 222-224). Di Celle et al teach that IL-8 mRNA expression is increased in a variety of cancer types (e.g., page 226, left column), and that evaluation of cytokine production can help to better understand processes that govern proliferation and growth (e.g., page 227, left column, 1st full paragraph).

GenBank Accession No. XM_031289 teaches the sequence of human IL-8 transcript. This sequence is identical to instant SEQ ID NO: 1 (see the attached alignment in Appendix II).

Because Kanaoka et al teach amplifying and quantifying Cox-2 RNA expression in human cells, and GenBank Accession No. XM_051900 teaches the Cox-2 sequence transcribed in humans, it would have been within the skill of the art to substitute the specific sequence of XM_051900 for the sequence used by Kanaoka et al in order to achieve the predictable result of amplifying and quantifying Cox-2 RNA expression in human cells. Furthermore, because Kanaoka et al teach the detection of the amplified cDNA product of COX-2 by gel electrophoresis, and di Celle et al teach the detection of amplified cDNA by gel electrophoresis in a gel stained with ethidium bromide, a chromophore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to include ethidium bromide in the gel used to detect COX-2 amplified cDNA in order to achieve the expected result of being able to visualize the amplified cDNA in the gel.

Furthermore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the assay of Kanaoka et al to include the measurement of IL-8 RNA expression in the colorectal samples, as taught by Brew et al, using the method of di Celle et al. Kanaoka et al teach it is within the ordinary skill in the art to use RT-PCR to detect RNA expression of a gene increased in expression in colorectal cancer as compared to normal colorectal cells, and Brew et al teach the quantification of IL-8 RNA expression in colorectal cancer and normal colorectal mucosa. Furthermore, di Celle et al teach the quantification of IL-8 mRNA expression by RT-PCR. Thus, it would have been obvious to combine the quantification of Cox-2 RNA expression and IL-8 RNA expression in the same samples using the same type of RT-PCR assay. Moreover, it would have been obvious to one of ordinary skill in the art to substitute the specific sequence of XM_031289 for the sequence of IL-8, because both Brew et al

and di Celle et al teach the measurement of IL-8 expression in human cells, and XM_032189 provides the sequence of the human IL-8 transcript.

One would have been motivated to make such a modification in order to receive the expected benefit of furthering the understanding of IL-8 expression in colorectal carcinoma and normal mucosa as taught by Brew et al, and suggested by di Celle et al with regard to proliferative disease and cancer. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 50, 51 and 99 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kanaoka et al (Japanese Journal of Gastroenterology, Vol. 99, No. 44taikai, page A634, Abstract P-379, September 20, 2002; see the entire reference and attached translation) in view of GenBank Accession No. XM_051900 (GI: 14730543, publicly available July 2001; see the entire reference), Brew et al (European Journal of Cancer, Vol. 32A, No. 12, pages 2142-2147, 1996; see the entire reference), di Celle et al (Blood, Vol. 84, No. 1, pages 220-228, July 1994; see the entire reference), and GenBank Accession No. XM_031289 (GI: 14731054, publicly available July 2001; see the entire reference) as applied to claims 49, 54, 55, 57, 61, 63, 64, 96, 98, 100-103, 105 and 106 above, and further in view of DuBois et al (Carcinogenesis, Vol. 19, No. 1, pages 49-53, 1998; see the entire reference), Park et al (Diabetes, Vol. 46, No. 7, pages 1230-1234, July 1997; see the entire reference) and GenBank Accession No. XM_003059 (GI: 13646004, publicly available April 2001; see the entire reference). This is a new rejection.

The combined teachings of Kanaoka et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, and GenBank Accession No. XM_031289 are described above and applied as before.

Kanaoka et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, and GenBank Accession No. XM_031289 do not teach the method further comprising amplifying and quantifying RNA expression, and comparing the quantified expression levels in a colorectal sample from a subject and a normal control colorectal sample for SEQ ID NO: 16 (PPARG).

DuBois et al teach that they previously demonstrated increased COX-2 expression in human colorectal adenocarcinomas when compared to normal adjacent colonic mucosa, and these findings have been confirmed by other investigators who have shown elevated levels of COX-2 protein in colorectal tumors by immunoblotting, and immunohistochemical staining (e.g., page 49, right column, full paragraph). DuBois et al teach that COX-2 mRNA and protein expression has also been found to be increased in intestinal tumors that develop in rodents following carcinogen treatment and in adenomas taken from *Min* mice (e.g., page 49, right column, full paragraph). DuBois et al teach that COX-2 overexpression in rat intestinal epithelial cells leads to phenotypic alterations, such increase tumorigenic potential, and the phenotypic alterations can be reversed by treatment with highly selective COX-2 inhibitors (e.g., paragraph bridging page 49-50). DuBois et al teach that the eicosanoid products formed by the COX-2 enzyme are likely affecting downstream signaling pathways, ultimately regulating gene transcription, and one candidate for eicosanoid mediated transcriptional regulation is the PPARγ nuclear receptor (e.g., paragraph bridging pages 49-50). Based upon this rationale, DuBois et al undertook studies to determine if PPARγ is aberrantly expressed in colon tumor cells and found

that PPARγ mRNA and protein was expressed in intestinal tumor, and a subset of polyps and human colon cancer cell lines (e.g., paragraph bridging pages 49-50; page 50, paragraph bridging columns; page 51, right column, 1st full paragraph). Figure 1 shows the expression of PPARγ and COX-2 in normal control colorectal tissue and colorectal tumor tissue from rats. Based upon the data from rats, DuBois et al hypothesized that PPARγ would be expressed in human colon cancer cells as well (e.g., page 51, right column, 1st full paragraph). DuBois et al measured the expression of PPARγ in the human colon cancer cell lines and determined that some of the cells lines express PPARγ (e.g., page 51, right column, 1st full paragraph; Figure 4). DuBois et al teach that work was underway to determine the biological relevance of aberrant co-expression of PPARγ and COX-2 (e.g., page 52, paragraph bridging columns).

Park et al teach it is within the skill of the art to measure increased PPARγ mRNA expression in human cells by RT-PCR (e.g., pages 1231-1232, Reverse transcription-polymerase chain reaction (RT-PCR) of PPAR-γ). Further, Park et al teach that the tissue specificity, relative abundance, and regulation of expression of PPARγ in human tissues need to be defined to address the physiological function of PPARγ (e.g., page 1230, right column).

GenBank Accession No. XM_003059 teaches the sequence of human PPARγ transcript. This sequence is identical to instant SEQ ID NO: 16 (see the attached alignment in Appendix III).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the combined teachings of Kanaoka et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, and GenBank Accession No. XM_031289 to include the quantitative RT-PCR assay for PPARγ taught by Park et al in the normal and cancerous

colorectal tissue. DuBois et al teach a potential relationship between COX-2 RNA expression and PPARγ RNA expression in colorectal cancer, and Park et al teach it is desirable to quantitate PPARγ RNA expression to address the physiological function of PPARγ. Furthermore, it would have been obvious to one of ordinary skill in the art to substitute the specific sequence of XM_003059 for the sequence of PPARγ, because both DuBois et al and Park et al teach the measurement of PPARγ expression in human cells, and XM_003059 provides the sequence of the human PPARγ transcript.

One would have been motivated to make such a modification in order to receive the expected benefit of better understanding the expression of PPARγ RNA in colorectal cells obtained from the feces of subjects with colorectal cancer and subjects with no organic colorectal disease, as it relates to Cox-2 RNA expression in the same cells. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 52 and 53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kanaoka et al (Japanese Journal of Gastroenterology, Vol. 99, No. 44taikai, page A634, Abstract P-379, September 20, 2002; see the entire reference and attached translation) in view of GenBank Accession No. XM_051900 (GI: 14730543, publicly available July 2001; see the entire reference), Brew et al (European Journal of Cancer, Vol. 32A, No. 12, pages 2142-2147, 1996; see the entire reference), di Celle et al (Blood, Vol. 84, No. 1, pages 220-228, July 1994; see the entire reference), and GenBank Accession No. XM_031289 (GI: 14731054, publicly available

July 2001; see the entire reference), and further in view of DuBois et al (Carcinogenesis, Vol. 19, No. 1, pages 49-53, 1998; see the entire reference), Park et al (Diabetes, Vol. 46, No. 7, pages 1230-1234, July 1997; see the entire reference) and GenBank Accession No. XM_003059 (GI: 13646004, publicly available April 2001; see the entire reference) as applied to claims 49-51, 54, 55, 57, 61, 63, 64, 96, 98-103, 105 and 106 above, and further in view of Baker et al (WO 2003/078662 A1, cited in a prior action; see the entire reference) and Gould et al (Kidney International, Vol. 61, pages 51-60, January 2002, cited in a prior action; see the entire reference). This is a new rejection.

The teachings of Kanaoka et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, GenBank Accession No. XM_031289, DuBois et al, Park et al, and GenBank Accession No. XM_003059 are described above and applied as before. Further, it was known that the RT-PCR process taught by Kanaoka et al necessarily involves the use of enzymes and reagents for the preparation of cDNA (i.e., the reverse transcription (RT) of RNA to cDNA) (e.g., di Celle et al. page 221, right column, *RT-PCR*).

Kanaoka et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, GenBank Accession No. XM_031289, DuBois et al, Park et al, and GenBank Accession No. XM_003059 do not teach the method where the step of amplifying further comprises using primers of SEQ ID NO: 47 and 48 for Cox-2, and primers of SEQ ID NO: 45 and 46 for IL-8.

Baker et al teach a panel of two or more gene specific primers selected from the group consisting of the forward and reverse primers listed in Table 2 (e.g., page 18, lines 3-4). Table 2 contains forward and reverse primers for COX2 (PTGS2), which consist of SEQ ID NOs: 229 and 230 (e.g., Table 2 at page 72). The sequences of Baker et al, SEQ ID NOs: 229 (5'-

TCTGCAGAGTTGGAAGCACTCTA-3') and 230 (5'-GCCGAGGCTTTTCTACCAGAA-3') consist of sequences 100% identical to the claimed sequences of SEQ ID NOs: 47 and 48 (e.g., page 29 of the sequence listing of Baker et al). Baker et al teach that the primers may be used for gene expression profiling using RT-PCR preceded by an amplification step (e.g., page 5, lines 22-27; page 7, lines 7-9 and 25). Further, Baker et al teach RT-PCR of IL8 (e.g., page 8, lines 31-33). Baker et al teach further reagents for RT-PCR, including reagents for the preparation of cDNA, such as the GeneAmp RNA PCR kit; and reagents for the detection and quantitation of polynucleotides that contain at least one chromophore, such as components for TaqMan PCR® where the probe is designed to detect a nucleotide sequence between the two primers and is labeled with a reporter fluorescent dye (e.g., pages 31-32). Baker et al teach that RT-PCR is a flexible and quantitative method that can be used to compare mRNA levels in different sample populations, tumor tissues, including colon cancer, and corresponding normal tissues to characterize patters of gene expression (e.g., page 4, lines 14-18; page 5, lines 18-21; page 7, lines 12-16; page 31, lines 12-18; page 31, lines 8-11).

Gould et al teach oligonucleotides to be used as primers for RT-PCR of IL8 (e.g., Table 1). The forward primer for IL8 is 5'-AGATATTGCACGGGAGAATATACAAA-3', and the reverse primer for IL8 is 5'-TCAATTCCTGAAATTAAAGTTCGGATA-3' (Table 1). The primer sequences taught by Gould et al consist of a nucleic acid sequence 100% to SEQ ID NOs: 45 and 46.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the combined teaches teachings to include the specific primer sequences taught by Baker et al and Gould et al because Kanaoka et al, di Celle et al and Baker et al teach it

is within the ordinary skill in the art to use primers for RT-PCR to quantify the RNA expression levels of Cox-2 and IL-8.

One would have been motivated to make such a modification in order to receive the expected benefit of using primers known in the art to successfully amplify Cox-2 and IL-8 as taught by Baker et al and Gould et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 49, 54, 55, 57, 96, 98 and 100-102 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hao et al (Journal of Pathology, Vol. 187, pages 295-301, 1999; see the entire reference) in view of GenBank Accession No. XM_051900 (GI: 14730543, publicly available July 2001; see the entire reference), Brew et al (European Journal of Cancer, Vol. 32A, No. 12, pages 2142-2147, 1996; see the entire reference), di Celle et al (Blood, Vol. 84, No. 1, pages 220-228, July 1994; see the entire reference), and GenBank Accession No. XM_031289 (GI: 14731054, publicly available July 2001; see the entire reference). This is a new rejection.

For the purpose of this rejection, independent claims 98 and claims that depend therefrom have been interpreted as being directed to a method of assessing the risk of colorectal cancer (see the rejection under 35 U.S.C. 112, second paragraph).

Hao et al teach that accumulating evidence suggested that both human and rodent colorectal tumor tissues contain elevated mRNA or protein levels of Cox-2 (e.g., page 295, paragraph bridging columns). Hao et al teach the collection of 85 sporadic colorectal adenomas, 53 colorectal carcinomas, and 19 samples of paired tumor and adjacent grossly normal mucosa

(e.g., page 296, left column, 1st and 2nd full paragraph). Hao et al teach selecting Cox-2 as a biomarker; isolating RNA from the samples; and amplifying and quantifying Cox-2 RNA expression by RT-PCR (e.g., page 296, left column, 2nd full paragraph; page 297, left column, 2nd and 3rd full paragraphs; page 297, paragraph bridging columns). Hao et al teach that the step of amplifying further comprises using enzymes and reagents for the preparation of cDNA (e.g., page 297, left column, 3rd full paragraph). Hao et al teach that the step of quantifying the levels of RNA further comprises labeling the amplified polynucleotide with ethidium bromide, which is a chromophore, in an agarose gel, and visualizing the amount of product using ultraviolet fluorescence (e.g., page 297, paragraph bridging columns). Further, Hao et al teach comparing the quantified expression levels of Cox-2 mRNA in a colorectal adenoma sample or a colorectal carcinoma sample to a normal control colorectal sample (e.g., page 298, right column, full paragraph; Table IV). Hao et al teach determining a difference in the expression levels of the colorectal adenoma sample as compared to the normal control, and determining a difference in the expression levels of the colorectal carcinoma sample as compared to the normal control, where significantly increased expression of Cox-2 is seen colorectal adenomas and carcinomas as compared with normal mucosa (e.g., page 298, right column, full paragraph; Table IV). Hao et al conclude the Cox-2 up-regulation occurs at an early stage in colorectal tumorigenesis, thus indicating an increased risk of transformation (e.g., page 295, paragraph bridging columns; page 299, paragraph bridging columns).

Hao et al do not teach that Cox-2 has the sequence of SEQ ID NO: 2. Hao et al do not teach amplifying, quantifying and comparing the expression of SEQ ID NO: 1 (IL-8) in the colorectal samples.

GenBank Accession No. XM_051900 teaches the sequence of human Cox-2 (a.k.a. prostaglandin-endoperoxide synthase 2). This sequence is identical to instant SEQ ID NO: 2 (see the attached alignment in Appendix I).

Brew et al teach that IL-8 production was known to be a feature of certain human tumor cell lines derived from colon carcinoma (e.g., page 2143, left column, 1st paragraph). Brew et al teach the collection of tissue samples of normal colon, neoplastic colorectal and adjoining non-involved tissue, and lymph node metastasis (e.g., page 2143, left column, 2nd paragraph). Brew et al teach the construction of sense and antisense riboprobes to the IL-8 cDNA for use in *in situ* hybridization to quantify the level of IL-8 RNA expression in the collected tissue samples (e.g. page 2143, left column, 4th paragraph and right column, 1st paragraph). Brew et al teach that all 10 adenocarcinoma samples, including a lymph node metastasis, showed cytoplasmic hybridization with the IL-8 probe, and the intensity of staining ranged from weak to strong (e.g., paragraph bridging pages 2143-2144; Table 1). Further, Brew et al compared the level of IL-8 RNA expression in the adenocarcinomas to the expression in normal colonic mucosa, and determined that expression was increased in the adenocarcinoma relative to the normal colonic mucosa, which showed much weaker staining or was completely negative (e.g., paragraph bridging pages 2143-2144).

Di Celle et al teach a sensitive reverse-transcriptase polymerase chain (RT-PCR) analysis performed to evaluate the expression of a panel of cytokine mRNAs in unstimulated B-LL cells, where the panel includes IL-8 (e.g., page 220, paragraph bridging columns). Di Celle et al teach RT-PCR of IL-8 from total cellular RNA followed by analysis in gels stained with ethidium bromide (e.g., page 221, right column, full paragraph). Di Celle et al teach that the IL-8 mRNA

can be quantified relative to a co-amplified gene (e.g., paragraph bridging pages 222-224). Di Celle et al teach that IL-8 mRNA expression is increased in a variety of cancer types (e.g., page 226, left column), and that evaluation of cytokine production can help to better understand processes that govern proliferation and growth (e.g., page 227, left column, 1st full paragraph).

GenBank Accession No. XM_031289 teaches the sequence of human IL-8 transcript. This sequence is identical to instant SEQ ID NO: 1 (see the attached alignment in Appendix II).

Because Hao et al teach amplifying and quantifying Cox-2 RNA expression in human cells, and GenBank Accession No. XM_051900 teaches the Cox-2 sequence transcribed in humans, it would have been within the skill of the art to substitute the specific sequence of XM_051900 for the sequence used by Hao et al in order to achieve the predictable result of amplifying and quantifying Cox-2 RNA expression in human cells.

Furthermore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the assay of Hao et al to include the measurement of IL-8 RNA expression in the colorectal samples, as taught by Brew et al, using the method of di Celle et al. Hao et al teach it is within the ordinary skill in the art to use RT-PCR to detect RNA expression of a gene increased in expression in colorectal cancer as compared to normal mucosa, and Brew et al teach the quantification of IL-8 RNA expression in colorectal cancer and normal mucosa. Furthermore, di Celle et al teach the quantification of IL-8 mRNA expression by RT-PCR. Thus, it would have been obvious to combine the quantification of Cox-2 RNA expression and IL-8 RNA expression in the same samples using the same type of RT-PCR assay. Moreover, it would have been obvious to one of ordinary skill in the art to substitute the specific sequence of XM_031289 for the sequence of IL-8, because both Brew et al and di Celle et al teach the

measurement of IL-8 expression in human cells, and XM_032189 provides the sequence of the human IL-8 transcript.

One would have been motivated to make such a modification in order to receive the expected benefit of furthering the understanding of IL-8 expression in colorectal carcinoma, colorectal adenoma, and normal mucosa as taught by Brew et al, and suggested by di Celle et al with regard to proliferative disease and cancer. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 50, 51 and 99 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hao et al (Journal of Pathology, Vol. 187, pages 295-301, 1999; see the entire reference) in view of GenBank Accession No. XM_051900 (GI: 14730543, publicly available July 2001; see the entire reference), Brew et al (European Journal of Cancer, Vol. 32A, No. 12, pages 2142-2147, 1996; see the entire reference), di Celle et al (Blood, Vol. 84, No. 1, pages 220-228, July 1994; see the entire reference), and GenBank Accession No. XM_031289 (GI: 14731054, publicly available July 2001; see the entire reference) as applied to claims 49, 54, 55, 57, 96, 98 and 100-102 above, and further in view of DuBois et al (Carcinogenesis, Vol. 19, No. 1, pages 49-53, 1998; see the entire reference), Park et al (Diabetes, Vol. 46, No. 7, pages 1230-1234, July 1997; see the entire reference) and GenBank Accession No. XM_003059 (GI: 13646004, publicly available April 2001; see the entire reference). This is a new rejection.

The combined teachings of Hao et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, and GenBank Accession No. XM_031289 are described above and applied as before.

Hao et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, and GenBank Accession No. XM_031289 do not teach the method further comprising amplifying and quantifying RNA expression, and comparing the quantified expression levels in a colorectal sample from a subject and a normal control colorectal sample for SEQ ID NO: 16 (PPARG).

DuBois et al teach that they previously demonstrated increased COX-2 expression in human colorectal adenocarcinomas when compared to normal adjacent colonic mucosa, and these findings have been confirmed by other investigators who have shown elevated levels of COX-2 protein in colorectal tumors by immunoblotting, and immunohistochemical staining (e.g., page 49, right column, full paragraph). DuBois et al teach that COX-2 mRNA and protein expression has also been found to be increased in intestinal tumors that develop in rodents following carcinogen treatment and in adenomas taken from *Min* mice (e.g., page 49, right column, full paragraph). DuBois et al teach that COX-2 overexpression in rat intestinal epithelial cells leads to phenotypic alterations, such increase tumorigenic potential, and the phenotypic alterations can be reversed by treatment with highly selective COX-2 inhibitors (e.g., paragraph bridging page 49-50). DuBois et al teach that the eicosanoid products formed by the COX-2 enzyme are likely affecting downstream signaling pathways, ultimately regulating gene transcription, and one candidate for eicosanoid mediated transcriptional regulation is the PPARγ nuclear receptor (e.g., paragraph bridging pages 49-50). Based upon this rationale, DuBois et al undertook studies to determine if PPARγ is aberrantly expressed in colon tumor cells and found

that PPARγ mRNA and protein was expressed in intestinal tumor, and a subset of polyps and human colon cancer cell lines (e.g., paragraph bridging pages 49-50; page 50, paragraph bridging columns; page 51, right column, 1st full paragraph). Figure 1 shows the expression of PPARγ and COX-2 in normal control colorectal tissue and colorectal tumor tissue from rats. Based upon the data from rats, DuBois et al hypothesized that PPARγ would be expressed in human colon cancer cells as well (e.g., page 51, right column, 1st full paragraph). DuBois et al measured the expression of PPARγ in the human colon cancer cell lines and determined that some of the cells lines express PPARγ (e.g., page 51, right column, 1st full paragraph; Figure 4). DuBois et al teach that work was underway to determine the biological relevance of aberrant co-expression of PPARγ and COX-2 (e.g., page 52, paragraph bridging columns).

Park et al teach it is within the skill of the art to measure increased PPARγ mRNA expression in human cells by RT-PCR (e.g., pages 1231-1232, Reverse transcription-polymerase chain reaction (RT-PCR) of PPAR-γ). Further, Park et al teach that the tissue specificity, relative abundance, and regulation of expression of PPARγ in human tissues need to be defined to address the physiological function of PPARγ (e.g., page 1230, right column).

GenBank Accession No. XM_003059 teaches the sequence of human PPARγ transcript. This sequence is identical to instant SEQ ID NO: 16 (see the attached alignment in Appendix III).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the combined teachings of Hao et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, and GenBank Accession No. XM_031289 to include the quantitative RT-PCR assay for PPARγ taught by Park et al in the normal and cancerous colorectal tissue. DuBois et al

teach a potential relationship between COX-2 RNA expression and PPARγ RNA expression in colorectal cancer, and Park et al teach it is desirable to quantitate PPARγ RNA expression to address the physiological function of PPARγ. Furthermore, it would have been obvious to one of ordinary skill in the art to substitute the specific sequence of XM_003059 for the sequence of PPARγ, because both DuBois et al and Park et al teach the measurement of PPARγ expression in human cells, and XM_003059 provides the sequence of the human PPARγ transcript.

One would have been motivated to make such a modification in order to receive the expected benefit of better understanding the expression of PPARγ RNA in normal human colorectal tissue, human colorectal adenomas and human colorectal carcinoma, as it relates to Cox-2 RNA expression in the same cells. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 52 and 53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hao et al (Journal of Pathology, Vol. 187, pages 295-301, 1999; see the entire reference) in view of GenBank Accession No. XM_051900 (GI: 14730543, publicly available July 2001; see the entire reference), Brew et al (European Journal of Cancer, Vol. 32A, No. 12, pages 2142-2147, 1996; see the entire reference), di Celle et al (Blood, Vol. 84, No. 1, pages 220-228, July 1994; see the entire reference), and GenBank Accession No. XM_031289 (GI: 14731054, publicly available July 2001; see the entire reference), and further in view of DuBois et al (Carcinogenesis, Vol. 19, No. 1, pages 49-53, 1998; see the entire reference), Park et al (Diabetes, Vol. 46, No. 7, pages 1230-1234, July 1997; see the entire reference) and GenBank Accession No. XM_003059 (GI:

13646004, publicly available April 2001; see the entire reference) as applied to claims 49-51, 54, 55, 57, 96 and 98-102 above, and further in view of Baker et al (WO 2003/078662 A1, cited in a prior action; see the entire reference) and Gould et al (Kidney International, Vol. 61, pages 51-60, January 2002, cited in a prior action; see the entire reference). This is a new rejection.

The teachings of Hao et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, GenBank Accession No. XM_031289, DuBois et al, Park et al, and GenBank Accession No. XM_003059 are described above and applied as before.

Hao et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, GenBank Accession No. XM_031289, DuBois et al, Park et al, and GenBank Accession No. XM_003059 do not teach the method where the step of amplifying further comprises using primers of SEQ ID NO: 47 and 48 for Cox-2, and primers of SEQ ID NO: 45 and 46 for IL-8.

Baker et al teach a panel of two or more gene specific primers selected from the group consisting of the forward and reverse primers listed in Table 2 (e.g., page 18, lines 3-4). Table 2 contains forward and reverse primers for COX2 (PTGS2), which consist of SEQ ID NOs: 229 and 230 (e.g., Table 2 at page 72). The sequences of Baker et al, SEQ ID NOs: 229 (5'-TCTGCAGAGTTGGAAGCACTCTA-3') and 230 (5'-GCCGAGGCTTTTCTACCAGAA-3') consist of sequences 100% identical to the claimed sequences of SEQ ID NOs: 47 and 48 (e.g., page 29 of the sequence listing of Baker et al). Baker et al teach that the primers may be used for gene expression profiling using RT-PCR preceded by an amplification step (e.g., page 5, lines 22-27; page 7, lines 7-9 and 25). Further, Baker et al teach RT-PCR of IL8 (e.g., page 8, lines 31-33). Baker et al teach further reagents for RT-PCR, including reagents for the preparation of cDNA, such as the GeneAmp RNA PCR kit; and reagents for the detection and quantitation of

polynucleotides that contain at least one chromophore, such as components for TaqMan PCR® where the probe is designed to detect a nucleotide sequence between the two primers and is labeled with a reporter fluorescent dye (e.g., pages 31-32). Baker et al teach that RT-PCR is a flexible and quantitative method that can be used to compare mRNA levels in different sample populations, tumor tissues, including colon cancer, and corresponding normal tissues to characterize patters of gene expression (e.g., page 4, lines 14-18; page 5, lines 18-21; page 7, lines 12-16; page 31, lines 12-18; page 31, lines 8-11).

Gould et al teach oligonucleotides to be used as primers for RT-PCR of IL8 (e.g., Table 1). The forward primer for IL8 is 5'-AGATATTGCACGGGAGAATATACAAA-3', and the reverse primer for IL8 is 5'-TCAATTCCTGAAATTAAAGTTCGGATA-3' (Table 1). The primer sequences taught by Gould et al consist of a nucleic acid sequence 100% to SEQ ID NOs: 45 and 46.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the combined teaches teachings to include the specific primer sequences taught by Baker et al and Gould et al because Hao et al, di Celle et al and Baker et al teach it is within the ordinary skill in the art to use primers for RT-PCR to quantify the RNA expression levels of Cox-2 and IL-8.

One would have been motivated to make such a modification in order to receive the expected benefit of using primers known in the art to successfully amplify Cox-2 and IL-8 as taught by Baker et al and Gould et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 61, 62, 103 and 104 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hao et al (Journal of Pathology, Vol. 187, pages 295-301, 1999; see the entire reference) in view of GenBank Accession No. XM_051900 (GI: 14730543, publicly available July 2001; see the entire reference), Brew et al (European Journal of Cancer, Vol. 32A, No. 12, pages 2142-2147, 1996; see the entire reference), di Celle et al (Blood, Vol. 84, No. 1, pages 220-228, July 1994; see the entire reference), and GenBank Accession No. XM_031289 (GI: 14731054, publicly available July 2001; see the entire reference) as applied to claims 49, 54, 55, 57, 96, 98 and 100-102 above, and further in view of Melville et al (Journal of Clinical Pathology, Vol. 41, pages 1180-1186, 1988; see the entire reference), and Ristimäki et al (US Patent No. 6,416,961 B1; see the entire reference). This is a new rejection.

The combined teachings of Hao et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, and GenBank Accession No. XM_031289 are described above and applied as before.

Hao et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, and GenBank Accession No. XM_031289 do not teach the method where the colorectal cells are obtained by the use of a swab.

Melville et al teach that brush cytology (i.e., swabbing) through a colonoscope has been shown to have a high accuracy in the diagnosis of colorectal cancer and has been recommended in the diagnosis of colonic strictures (e.g., page 1180, paragraph bridging columns). Melville et al teach the collection of cytology brushings during colonoscopy either with a standard sheathed 10 mm x 3 mm reusable colonoscopy brush, or in some cases a disposable brush (e.g., page

1181, left column, 2nd full paragraph). For brushings taken through a rigid sigmoidoscope, a brush with a rounded end was designed which could easily be rotated on the mucosa and which brushed an area of about 3 cm^2 (e.g., page 1181, paragraph bridging columns). Melville et al teach that neoplastic changes can be observed in cells collected by cytology brushing (e.g., page 1185, right column, 4th paragraph).

Ristimäki et al acknowledge that recent studies show that Cox-2 is connected to colon carcinogenesis (e.g., paragraph bridging columns 1-2). Ristimäki et al teach that increased Cox-2 RNA expression can be detected in patient samples obtained as biopsies or brush samples, which are obtained during routine gastroscopy or gastric lavage combined with brush technique (e.g., column 3, lines 21-23). Ristimäki et al teach that the brush technique is well known in the art in routine gastric cytology, and the technique provides cell samples from the gastric mucosa for microscopic examination, and markers, such as Cox-2 RNA, may increase the sensitivity and specificity of the assay (e.g., column 3, lines 24-33). Ristimäki et al teach that Cox-2 mRNA can be conveniently detected from the brush samples using methods known in the art, such as RT-PCR (e.g., column 3, lines 13-38).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the combined teaches, such that sample collection of colorectal cells is performed by swabbing with a brush during colonoscopy or sigmoidoscopy as taught by Melville et al, because Melville et al teach it is within the ordinary skill in the art to use a brush to collect colorectal cells and Ristimäki et al teach the use of cells obtained by brushing to detect levels of Cox-2 RNA expression, which is consistent with the combined teachings of the references and goal of providing levels of Cox-2 expression to evaluate colorectal carcinoma.

One would have been motivated to make such a modification in order to receive the expected benefit of using a less invasive method as compared to the biopsy method taught by Hao et al to achieve the same result of providing cells for the analysis of RNA expression as taught by Ristimäki et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 61, 63, 64, 103, 105 and 106 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hao et al (Journal of Pathology, Vol. 187, pages 295-301, 1999; see the entire reference) in view of GenBank Accession No. XM_051900 (GI: 14730543, publicly available July 2001; see the entire reference), Brew et al (European Journal of Cancer, Vol. 32A, No. 12, pages 2142-2147, 1996; see the entire reference), di Celle et al (Blood, Vol. 84, No. 1, pages 220-228, July 1994; see the entire reference), and GenBank Accession No. XM_031289 (GI: 14731054, publicly available July 2001; see the entire reference) as applied to claims 49, 54, 55, 57, 96, 98 and 100-102 above, and further in view of Davidson et al (Cancer Epidemiology, Biomarkers & Prevention, Vol. 4, pages 643-647, September 1995; see the entire reference). This is a new rejection.

The combined teachings of Hao et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, and GenBank Accession No. XM_031289 are described above and applied as before.

Hao et al, GenBank Accession No. XM_051900, Brew et al, di Celle et al, and GenBank Accession No. XM_031289 do not teach the method where the colorectal cells are obtained by non-invasive collection of a stool sample.

Davidson et al teach that approximately one-sixth to one-third of normal adult colonic epithelial cells are shed every day (e.g., page 643, right column, 1st full paragraph). Davidson et al teach that the number of intact cells isolated from fecal material is lower than the number of total cells shed; thus, an enhanced detection system is required to amplify potential intermediate biomarkers of colon cancer (e.g., page 643, right column, 1st full paragraph). Davidson et al teach such an enhanced detection system, which requires the use of semi-quantitative "mimic" RT-PCR to detect the expression of genes with potential diagnostic value in the colon (e.g., page 643, right column, 1st full paragraph; pages 643-644, Materials and Methods). Davidson et al teach that this experimental approach provides a sensitive method for detection of mRNA isolated from feces containing exfoliated colonocytes and a noninvasive means for monitoring changes in this population of cells (e.g., page 643, right column, 1st full paragraph). Davidson et al teach that their analysis of protein kinase C mRNA expression in shed colonocytes was consistent with previous results obtained from scraped colonic mucosa (e.g., page 646, left column, last paragraph).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the combined teaches, such that sample collection of colorectal cells is performed by collecting a fecal (i.e., stool) sample as taught by Davidson et al, because Davidson et al teach that colorectal cells are normally shed into the feces, and it is within the skill of the art to isolate RNA from these cells, and measure gene expression using RT-PCR.

Further, Davison et al teach that the procedure may be applied to the quantitation of colon cancer biomarkers, which is consistent with the combined teachings of the references and goal of providing levels of Cox-2 RNA expression to evaluate colorectal carcinoma.

One would have been motivated to make such a modification in order to receive the expected benefit of using a less invasive method as compared to the biopsy method taught by Hao et al to achieve the same result of providing cells for the analysis of RNA expression as taught by Davidson et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Response to Arguments - 35 USC § 103

The rejections of claims 79 and 81-88 under 35 U.S.C. 103(a) are moot in view of Applicant's cancellation of the claims in the reply filed 2/12/2010.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached at 571-272-0951. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Jennifer Dunston/
Examiner
Art Unit 1636